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THE TAXONOMIC STATUS OF *BOMBUS ALBOANALIS* FRANKLIN AND ITS RELATIONSHIP WITH OTHER TAXA OF THE SUBGENUS *PYROBOMBUS* FROM NORTH AMERICA AND EUROPE (HYMENOPTERA: APIDAE)

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Abstract.—*Bombus alboanalis* Franklin, 1913, has either been regarded as a distinct species or has been synonymized with *B. frigidus* Smith 1854, from which it differs in coat color of the abdomen. However, both taxa are sympatric in Alaska and there is no evidence for intergradation from coat color variation. We surveyed 18 enzymes by vertical starch-gel electrophoresis and compared *B. alboanalis* with *B. frigidus* and other species of the subgenus *Pyrobombus.* We found that *B. alboanalis* differs from *B. frigidus* at two enzyme loci suggesting that these taxa are genetically separated. According to the enzyme survey *B. alboanalis* belongs to a group of very closely related *Pyrobombus* species that includes *B. frigidus, B. cingulatus* Wahlberg and *B. jonellus* (Kirby), and more distant *B. sitkensis* Nylander, *B. mixtus* Cresson, *B. pratorum* (L.), and *B. pyrenaeus* (Pérez). We present evidence that *B. alboanalis* may be conspecific with *B. jonellus.*

Key Words.—Insecta, Apidae, enzyme electrophoresis, bumble bee genetic relationships, biogeography

Bombus alboanalis is a bumble bee from Alaska that was described as a species by Franklin (1913). This taxon is poorly understood. Frison (1929) listed *B. alboanalis* as a distinct species, but Burks (1951), who based his compilation largely on manuscript notes by the late T. H. Frison, treated *B. alboanalis* as a variety of *B. frigidus* Smith. Hurd (1979) followed Burks (1951) in listing *B. alboanalis* as a variety of *B. frigidus.* A published revision, however, is not available.

Bombus alboanalis and *B. frigidus* are structurally identical but they differ in their coat color of the abdomen, which is white tailed in *B. alboanalis* and red tailed in *B. frigidus.* Polymorphism in coat color is very frequent among bumble bees. Several polytypic species of the New World [e.g., *B. occidentalis* Greene] and the Old World in particular [e.g., *B. terrestris* auct., *B. soroeensis* (Fabr.)] are red tailed in one subspecies and white tailed in another, and a great variety of intermediates can be found where subspecies overlap in their distribution. In contrast, we have seen many specimens of *B. alboanalis* and *B. frigidus* from sympatric populations in Alaska (unpublished data), but there is no evidence for intergradation. This suggests to us that both taxa are distinct species. We have therefore used data from enzyme electrophoresis as additional information to investigate whether these taxa are genetically separated in areas of sympatry.

Table 1. Numbers of specimens of *Bombus jonellus* species group studied electrophoretically and origin of material.

	California	British Columbia	Alberta	Alaska	Norway	Switzerland	Total
B. alboanalis				8			8
B. cingulatus					3		3
B. frigidus			10	5			15
B. jonellus					6	14	20
B. mixtus	6	3	3				12
B. pratorum						14	14
B. pyrenaeus						10	10
B. sitkensis	2	4					6

Material and Methods

Bombus alboanalis and *B. frigidus* were collected from sympatric populations near Fairbanks, Alaska in 1985, 1991 and 1992. Additional specimens of *B. frigidus* are from three sites in southern Alberta where *B. alboanalis* has not been recorded. We include for comparison electrophoretic data of Scholl et al. (1988) on *Pyrobombus* species and additional specimens of six species of the *B. jonellus* species group as summarized in Table 1. The specimens were shipped on dry ice and stored at −80°C until used for electrophoresis. In addition, specimens of both taxa occurring sympatrically at several sites in Alaska were studied morphologically.

Electrophoresis.—Depending on the enzyme to be analysed either thoracic muscle (T) or abdominal tissue (A) and one of the following three buffers were used for electrophoresis: TBE = Tris-borate-EDTA, pH 9.3; TC = Tris-citrate, pH 7.3; AC = citrate-N-(3-aminopropyl)-morpholine, pH 6.2. The tissue was homogenized in ten volumes of Tris buffer, 0.1 M, pH 8.0. 20 μl of supernatant fractions of homogenates were applied to vertical starch gels in Buchler instruments, using a slotformer for 15 slots. The starch gels (13% w/v) were prepared from a mixture of 25 g of Connaught starch and 35 g starch from the Institut des Sciences de l'Evolution (Montpellier, France). The electrophoresis was run at 4° C for 15–16 h, voltage applied was 8 V cm^{-1} (TBE buffer), 4 V cm^{-1} (TC buffer) and 3 V cm^{-1} (AC buffer).

The gels were sliced once or twice to provide two or three slices, each of which was stained for a different enzyme. The enzymes scored are (tissue and buffer system used in brackets): aconitase (A, TC), 2 loci: ACO-1 and ACO-2; arginine kinase (A, TBE), APK; hydroxybutyric dehydrogenase (A, TC), BDH; α-glycerophosphate dehydrogenase (T, TC), 2 loci: GPD-2 and GPD-3; glutamic-oxalo-acetic transaminase (A, AC), GOT-2; glutamic-pyruvic transaminase (T, AC), GPT; hexokinase (T, TC), HK-3; isocitrate dehydrogenase (T, AC), IDH; leucine aminopeptidase (T, AC), LAP; malate dehydrogenase (T, AC), 2 loci: MDH-1 and MDH-2; malic enzyme (T, AC), MOD; peptidase (A, TC), PEP; phosphoglucose isomerase (A, TBE), PGI; phosphoglucomutase (A, TC), PGM; superoxide dismutase (A, TBE), SOD. Total: 18 loci.

In this survey the enzymes scored were selected by quality of resolution rather than enzyme function. The enzyme staining followed standard procedures of our

laboratory (Scholl et al. 1978, Bulnheim & Scholl 1981, Geiger & Scholl 1985); most of these are originally from Ayala et al. (1972) and Harris & Hopkinson (1976) and were slightly modified for optimal staining. Agar overlays were used to detect the following enzymes: ACON, APK, GPT, HK, PEP, PGI and PGM. All zymograms were photographed (Polaroid) for reference. The designation of electromorphs is based on mobilities (in mm) relative to the electromorph of *B. lucorum* (= index 100) which was used as a reference as in previous electrophoretic studies on bumble bees (Scholl et al. 1990, 1992). A phenogram of the genetic relationships of the species investigated was constructed by average linkage cluster analysis (UPGMA) (Nei 1987) using Nei's (1972) genetic distance (D).

Specimens Analyzed.—Bombus alboanalis: ALASKA. *FAIRBANKS NORTH STAR BOROUGH:* nr. Fairbanks, 25 May 1990 & 25 Jun 1992.

Bombus frigidus: ALASKA. *FAIRBANKS NORTH STAR BOROUGH:* nr. Fairbanks, 25 Jun 1992. CANADA. *ALBERTA:* Ya-Ha Tinda Ranch, E boundary of Banff National Park, 51°43′ N, 115°30′ W, 28 May 1989; Barrier Lake, Kananaskis River Valley, 51°02′ N, 115°02′ W, 27 May 1989; Stettler, 8 km W, 22 May 1989.

In addition to the *B. alboanalis* and *B. frigidus* analyzed electrophoretically, we have examined morphologically specimens of both taxa occurring sympatrically at several other sites: ALASKA. *FAIRBANKS NORTH STAR BOROUGH:* Fairbanks, 5–7 Jun 1990; *MATANUSKA-SUSITNA BOROUGH:* Denali Highway, Mile 30, 15 & 21 Jul 1993, and Mile 82, 20 Jul 1993; *SOUTHEAST FAIRBANKS BOROUGH:* Walker Fork Campground, Klondike Loop D-94.7, Taylor Highway, 7 Jul 1978: *VALDEZ CORDOVA BOROUGH:* Paxson, 14 & 20 Jul 1993; *YUKON-KOYUKUK BOROUGH:* Camp Denali, McKinley Park, 12 Jun–2 Aug 1985; Kathul Mt., Yukon River 1 May–1 Jul 1991.

Results and Discussion

Among the sympatric specimens of *B. alboanalis* and *B. frigidus* we examined morphologically, we found no intergradation in color pattern between the two taxa.

Allele frequencies at eleven loci which showed polymorphism and/or interspecific variation are listed in Fig. 1. As in previous studies of Hymenoptera in general (Packer & Owen, 1992) and other bumble bee species in particular (Obrecht & Scholl 1981; Scholl et al. 1990, 1992) the level of polymorphism was very low in the present study. In fact, most samples were monomorphic in all loci, except *B. frigidus* from Alberta, where one specimen was heterozygous at the ACO-1 locus and two specimens from different sites were heterozygous at the HK-3 locus; one specimen of *B. pratorum* was heterozygous at the ACO-1 locus. A rather unusual situation, however, was observed in *B. jonellus,* where samples from both Norway and Switzerland were highly polymorphic at the HK-3 locus, three alles were observed in similar frequencies in the populations from both countries, as shown in Fig. 1. In addition, two specimens of *B. jonellus* from Switzerland were heterozygous at the PEP locus. Seven enzymes were invariant in all specimens surveyed. These enzymes (APK, BDH, GPD-2, GPD-3, MDH-2, PGI and SOD) are not shown in Fig 1.

The *B. alboanalis* and *B. frigidus* from sympatric populations in Alaska showed a consistent difference in HK-3 and PGM, resulting in the allele frequencies that

	ACO-1			ACO-2			GOT-2		GPT		HK-3					IDH			LAP		MDH-1		MOD		PEP		PGM	
	90	96	100	100	102	104	98	105	100	107	89	93	96	100	105	100	105	113	94	96	93	101	98	102	82	92	87	94
B. alboanalis		1.00				1.00	1.00			1.00			1.00			1.00				1.00	1.00			1.00		1.00		1.00
B. frigidus Alaska		1.00				1.00	1.00			1.00		1.00				1.00				1.00	1.00			1.00		1.00	1.00	
B. frigidus Alberta	0.05	0.95				1.00	1.00			1.00		0.90	0.10			1.00				1.00	1.00			1.00		1.00	1.00	
B. cingulatus		1.00				1.00	1.00			1.00	1.00					1.00				1.00	1.00			1.00		1.00	1.00	
B. jonellus Norway		1.00				1.00	1.00			1.00		0.59	0.33		0.08			1.00		1.00	1.00			1.00		1.00	1.00	
B. jonellus Switzerland		1.00				1.00	1.00			1.00		0.50	0.44		0.06			1.00		1.00	1.00			1.00	0.07	0.93	1.00	
B. sitkensis		1.00			1.00		1.00			1.00	1.00					1.00			1.00			1.00	1.00			1.00	1.00	
B. mixtus		1.00		1.00			1.00			1.00	1.00					1.00			1.00			1.00		1.00		1.00	1.00	
B. pratorum		0.97	0.03	1.00				1.00		1.00				1.00		1.00			1.00			1.00		1.00		1.00	1.00	
B. pyrenaeus		1.00		1.00				1.00	1.00		1.00						1.00			1.00		1.00		1.00		1.00	1.00	

*No variation: APK, BDH, GPD-2, GPD-3, MDH-2, PGI, SOD

Figure. 1. Allele frequencies at eleven enzyme loci that showed polymorphism and/or interspecific variation.

are shown in Fig. 1; all other enzymes were identical. The data on HK-3 and PGM are of particular interest because they show that sympatric *B. alboanalis* and *B. frigidus* have separate gene pools. The comparison of *B. frigidus* from Alaska and Alberta showed minor differences, two rare alleles which were found at the ACO-1 and HK-3 locus in Alberta were not observed in Alaska (Fig. 1). The *B. cingulatus* specimens differed from *B. frigidus* at the HK-3 locus, having an allele that was otherwise found in *B. sitkensis, B. mixtus* and *B. pyrenaeus* respectively (Fig. 1), although *B. jonellus* was different from *B. frigidus* in IDH, in addition to the HK-3 polymorphism which was mentioned above.

Clearly, more differences were observed between *B. sitkensis, B. mixtus, B. pratorum* and *B. pyrenaeus* (Fig. 1) and in comparisons of these with the other listed taxa. These differences need not be presented in detail here. We summarize the genetic differentiation among the taxa surveyed in a phenogram (Fig. 2), that was generated by average linkage cluster analysis (UPGMA), using Nei's standard genetic distance that was calculated from all 18 loci surveyed. This phenogram suggests that *B. alboanalis, B. frigidus, B. cingulatus* and *B. jonellus* are very closely related, but *B. sitkensis, B. mixtus, B. pratorum* and *B. pyrenaeus* are more distant.

Regarding the relationships of *B. alboanalis* to other bumble bees, Franklin (1913: 387) wrote: "I have seen a few workers of *B. montanus* Lep., determined by Gerstaecker, and that species seems to be very closely allied to *alboanalis,* and it is possible that they should be considered as variations of the same species." *Bombus montanus* (Lepeletier), 1836, is now a synonym of *B.* (*Thoracobombus*) *ruderarius* (Müller), 1776, and is regarded as a subspecies (cf. Rasmont, 1983). However, Gerstaecker (1869) apparently confused other species with *B. montanus* Lep.; his *B. montanus* is now both in the synonymy of *B.* (*Melanobombus*) *sicheli* Kriechbaumer, 1873 (Dalla Torre 1896) and *B.* (*Pyrobombus*) *pyrenaeus* Pérez, 1879 (Tkalcu 1969). Considering these facts, it is not possible to decide what Franklin really had for comparison. *Bombus ruderarius montanus, B. sicheli alticola* and *B. pyrenaeus* clearly differ from *B. alboanalis* in the coloration of the abdomen which is red tailed in the former three taxa and white tailed in *B. alboanalis.* However, Franklin (1913: 387) must have had white tailed specimens for comparison, as he wrote: "the only difference in coloration of pile is that the black interalar band of *montanus* is wider than that of *alboanalis.*"

The close genetic relationships of the nearctic *B. alboanalis* and *B. frigidus* and the palaearctic *B. cingulatus* and *B. jonellus* that are revealed in this study, and in particular the low level of genetic differentiation between *B. alboanalis* and *B. jonellus* are of interest. In North America, *B. alboanalis* has been recorded from Alaska and Manitoba, Canada (Hurd 1979). One of us (RWT) has identified additional specimens from British Columbia and Yukon Territory. Franklin (1913) mentions Bering Island and Copper Island as Asian records. *Bombus frigidus* has a wider range. It is most common in Alaska and the Northwest Territories and is sparsely distributed along higher elevations of the continental divide, apparently as far south as Colorado (Stephen 1957, Hurd 1979). In contrast, *B. cingulatus* and *B. jonellus* have a very wide range in the palaearctic region. *Bombus cingulatus* is recorded from northern Scandinavia, east to Kamchatka, and to the Gulf of Anadyr (Reinig 1936, 1939). *Bombus jonellus* has a similar range in the north but extends west to Iceland (Prys Jones et al. 1981) and south to the Pyrenees

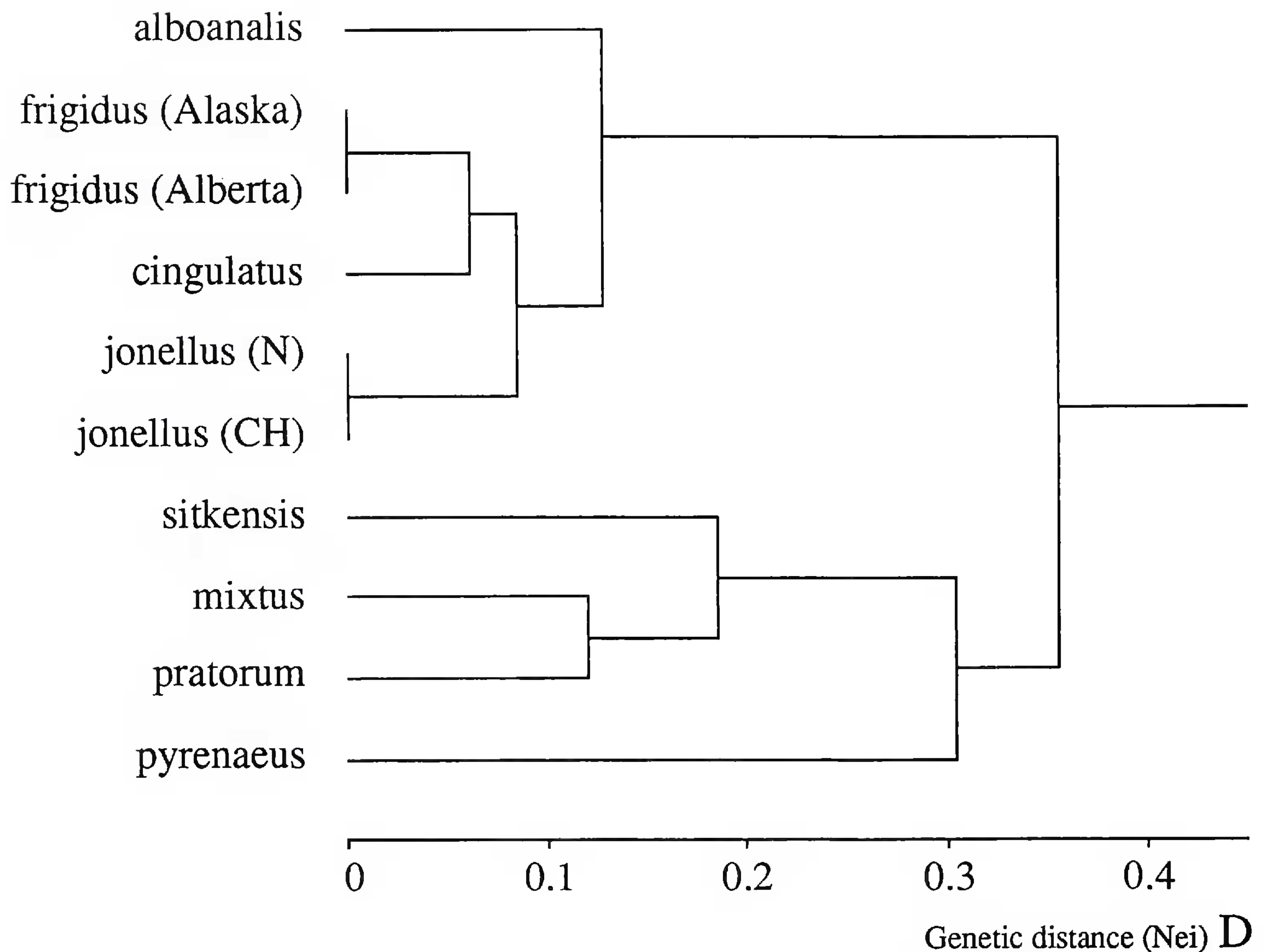

Figure. 2. Phenogram showing the genetic relationship between *B. alboanalis* and other taxa of the subgenus *Pyrobombus,* as revealed by the electrophoretic data.

and the Balkan (Rasmont 1983) and, in Asia, has even been recorded from the western Aleutian Islands (Panfilov 1982). Thus, *B. jonellus* and *B. alboanalis* are parapatric.

Bombus jonellus is a polytypic species (cf. Richards 1933, Rasmont 1983). Our electrophoresis survey covers the subspecies *B. j. martes* Gerstaecker, 1869 (specimens from Switzerland) and *B. j. subborealis* Richards, 1933 (specimens from Scandinavia). These taxa were identical in their enzyme patterns (Fig. 1). However, considering the very wide range of this species, one might expect electrophoretically detectable genetic differentiation within the range of this species even if levels of enzyme polymorphism are very low, as commonly observed in bumble bees (e.g., Obrecht & Scholl 1981, Pamilo et al. 1984, Scholl et al. 1990). Unfortunately, *B. jonellus* specimens from Siberia have not been available for electrophoretic studies, but we speculate that future studies might show that *B. alboanalis* is conspecific with *B. jonellus.* Specimens of *B. jonellus* and *B. cingulatus* from a transect from Siberia to Scandinavia would be needed to determine whether the electrophoretic pattern of *B. alboanalis* grades into those of European populations.

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